Characterization and bioprospects of silk protein from Spiders of Semi-Arid habitat

A

Synopsis

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1.0. INTRODUCTION

Spiders are found in almost all continent in the world except Antarctica. The seventh largest and oldest order of the animal kingdom is Aranea which include spiders. Recently 48,393 species belong to 4153 genera and 120 families were recorded from all over world **(world spider catalog 2020 version)**. In India there are 1686 species belong to 60 families and 438 genera were recorded **(Keshwani *et al*., 2012)** and in the semi-arid habitat about 83 species of Aranae have been recorded **(Anjali and Prakash, 2015)**. Spider dragline silk is the strongest and toughest biomaterial with the combination of tensile and elastic characteristics. Spider dragline has tensile strength which is comparable to Kevlar so spider use dragline silk in the web because it is strong yet flexible structure element in the web. Spider silk is for instance a promising tool with extensive usability in medical devices. In the middle ages spider webs were used as wound dressing-some reviews are even dated lower back to historic. The unmatched toughness of spider silk wound permit to improve several clinical products together with wound closure system, band-aids, and extraordinarily thin sutures for neurosurgery **(Scheibel, 2004)**. Moreover, spider silks may be in addition used in synthetic ligaments and tendons for durable implants. High performance fibres built from spider silks may be employed in numerous technical and business applications. In addition to uniqueness ropes and fishing nets, spider silk can be utilized for parachutes, ballistic applications (body armor), sporting goods, textiles and light-weight constructions for airplanes **(Gerritsen, 2002)**. Scientist in India have produced recombinant spider silk protein from the transgenic goat milk. In Tamil Nadu there has been work done on spider silk structure, properties and spinning.

There are 7 different types of spider silk for different purposes such as for capturing prey, for protection their new born, for locomotion or movement, for reproduction. The types of spider silk are aciniform, major ampullate, pyriform and tubuliform silk. Spider silk is secreted from the silk gland which are located in the abdomen. Silk genes are expressed in silk glands.

Spidroins are proteins which are encoded by spider silk gene (**Sandra M *et al*., 2018**). There are two types of protein of dragline silk are major ampullate spidroins 1and 2 i.e.Masp1 and Masp2 **(Sponner A *et al.*, 2005)**. Both silks are approximately 3500 amino acid long. MaSp1 may be found in the fibre middle and the periphery, whereas MaSp2 forms clusters in certain center areas. The large central domain names of MaSp1 and MaSp2 are organized in block copolymer-like

arrangements, in which two basic sequences, crystalline [poly (A) or poly (GA)] and much less crystalline (GGX or GPGXX) Polypeptides change in middle domain. The primary difference among MaSp1 and MaSp2 is the presence of proline (P) residues accounting for 15% of the whole amino acid content in MaSp2 **(Hu *et al*., 2006)**.

Due to various and unique properties of dragline silk it is used for drug delivery in medical application **(Altman GH *et al*., 2003)**, for tissue engineering **(Lewis RV *et al*., 2006)**, for cosmetic products and industrial material **(Tokareva *et al*., 2013)**.

Genetically engineered recombinant spider silk is not easy to produce because as it cannot be cultured like the silk worm as by nature, spiders are cannibalistic. New way of prospecting for the production of spider silk is required. *Nephila clavipes* genome size s predicted 3.45 GB with 55% estimated as repetitive sequence. To determine gene locations within the *N. Clavipes* genome, we sequenced RNA from 16 different tissue isolates (for example, whole body, brain and individual silk and venom glands) accured from 4 female individuals and then de novo assembled the transcriptome for each iolate using strand-precise 100-bp paired-stop reads **(Babb, 2017)**.

The present study will intend to focus on identifying the diversity of spiders which produce good quantity of silk. These silk will be extracted from the selected spider webs and quantitative and qualitative estimated.

2.0. REVIEW OF LITERATURE

Good work has been done on properties, structure and biotechnological application of spider silk, variety of the reviewed literature cited:

2.1. Review of literature on the diversity of spider and spider webs in semi-arid habitat

The first extensive studies on the diversity of spiders in areas with crop, non-crop and forest area of Agra region began with:

Anjali and Prakash (2012) and a total 34 species belonging to 12 families were recorded. Maximum number of spider species were from family - Salticidae, Oxyopidae, Lycosidae, Araneidae and Pholcidae and the major spiders observed were “orb-web builders”, “ground dwellers”, “sheet-web builders” whose occurrence ratio was 5:28:1. **Nogueira and Ades (2012)** also investigated the changes on the web construction by the spider *Argiope argentata* due to past experience spider web construction

behavior an improved by changing the phase from vertical phase (control phase) to horizontal phase. In the result due to the improvement in the horizontal periods so there is change in the spider web construction in the early stages.

Kumari *et al*., (2017) studied the diversity of spider fauna in semi-arid region of Rajasthan (Ajmer and Jodhpur). Maximum number of spiders are found and identify in woodland habitat are about 31.86 relative abundance and minimum number of spiders are found in caves/crevices/rocky area about 17.61 relative abundance. Araneidae and Oxyopidae were found as most abundant families with 41.44 and 15.78 percent of total spider fauna.

Patel and Patel (2018) studied that web construction pattern of spiders at District Mehsana situated in the northern area of Gujarat state. **Anjali and Prakash (2015, 2019)** Spiders have always been underestimated as model for research; however, they have provided unique behavior patterns in course of studies in their feeding, breeding, web building process and social aspects. It highlights female spiders have active role in the adaptation process.

2.2. Review of literature on the silk proteins of spiders and biotechnology applications

Fahnestock and Irwin (1997) studied the understanding the structural language of protein polymers and production of silk protein in *Escherichia coli.*

Scheibel (2004) studied on the designing of synthetic spider silk protein, assembly of recombinant spider silk, production of recombinant of spider silk protein, artificial spinning of spider silk and application for spider silk.

This was followed by the work of **Rising *et al*., (2005)** who reviewed about the spider silk and its assembly process and he also discussed about the application of spider silk in the biomedicine and materials engineering. **Zschokke and Herberstein (2005)** describes the thanks to obtain spiders by capturing them or by raising them from cocoons, the thanks to keep and feed spiders within the laboratory and therefore the thanks to encourage them to form webs. Finally it describes the thanks to document and analyze web building and web structure. **Römer and Scheibel (2008)** reviewed further outlines recent progress in recombinant production of spider silk proteins and their assembly into distinct polymer materials as a basis for novel products. **Teulé *et* al., (2009)** describes the protocol of silk gene design and cloning, protein expression in bacteria, recombinant protein purification and fiber formation.

Xia Xi *et al*., (2010) studied on the recombinant silk protein of the spider *Nephila clavipes* is produced in the metabollicaly engineered *E.coli which* result in a strong fibre comparable to native silk.

Eisoldt *et al*., (2011) studied this understanding of spider silk and its assembly process, further as discuss the applying of silk-based materials to the fields of biomedicine and materials engineering. **Chung *et al*., (2012)** reviewed recent development in production of spider silk proteins in various heterologous host systems. However, because of the difficulties in its production using spiders, alternative host systems and engineering methods are investigated the methods for developing suitable process that can produce spider silk protein. **Singh *et al*., (2012)** reviewed work was discuss about the gathering, composition, spinning and molecular structure, mechanical properties with its application of the spider silk.

Tokareva *et al*., (2013) studied on the current development in genetic engineering for the recombinant DNA production of spider silk protein in heterologous hosts for the various purposes like biomaterials and material science application.

Wohlrab *et al*., (2014) an summary on recent developments concerning gene-splicing and chemical modification of recombinant silk proteins are given, outlining the potential provided by recombinant spider silk chimeric proteins and spider silk-inspired polymers (combining synthetic polymers And spider silk peptides). **Anjali and Prakash (2015)** activity and expression levels of alcohol dehydrogenase, glucose-6-phosphate dehydrogenase and non-specific soluble esterase were compared within the seven species (C. lyoni, Hersilia sp. Oxyopes sp., Menemerusbivittatus, Leucaugedecorata, Selenops sp. and Cyrtophoracitricola) from the semi-arid habitat of Agra region.

Babb *et al*., (2017) find evidence of an alternatively spliced spidroin, a spidroin expressed only in venom glands, evolutionary mechanisms for spidroin diversification, and non-spidroin genes with expression patterns that suggest roles in silk production. **Blamires *et al*., (2017)** assimilated the research done and assess the techniques accustomed determine distinct varieties of spider silk chemical and property variability. We propose that more research should target testing hypotheses that designate spider silk property variations in ecological and evolutionary contexts. **Zoya and Kulkarni (2018)** reviewed Spider goat are the goats which contains spider genes which they lactate silk of spiders in their milk. Adam Rutherford modified the goat genetically during a farm which is found at the Utah State University and

folks goats produce ample amount of silk of spider in their milk, it constitute jointly of the strongest substances which are known to man.

Sandra M *et al*., (2018) studied the Characterization of *T. perfuga* silk genes, particularly CrSp, expand the range of the spidroin family and encourage new structure/function hypotheses.

3.0. OBJECTIVES OF THE STUDIES

- Identification of Spiders which produce large quantity of web silk protein.
- Qualitative analysis of silk protein.
- Quantitative estimation of web silk protein.
- Extraction of known gene locus of gene sequence of spider.
- Prospecting for producing recombinant spider silk web protein with *microbes.*

4.0. PROTOCOLS

4.1. Collection and Identification:

- Spiders will be collected by different methods like: Hand collection, Visual Searching, Inverted Umbrella, Pitfall meyhod.
- Spider build webs in shrubs, trees, long rock walls, storage rooms and in corners.
- Identify the silk protein producing spider on the basis of construction and quantity of web and their size.

4.2. Proteomic analysis:

- Qualitative analysis of Protein will be done by- SDS-PAGE Electrophoresis, 2D electrophoresis and Malditoff **(Abdullah *et al*., 2021)**.
- Standard Bradford assay for Quantitative estimation of Protein will be **(Abdullah *et al*., 2021)**.

4.3. Molecular genetic analysis:

- Standard available insect DNA kit will be utilized for the extraction of DNA.
- Amplification of the sequences through PCR followed by DNA sequencing of the identified gene **(Blake *et al*., 2016)**.

4.4. Biotechnological Production of Recombinant DNA:

- Standard method of **Teulé *et al*., (2009)** will be adapted initially for spider silk protein production in microbes *(E coli).*

PLAN OF WORK

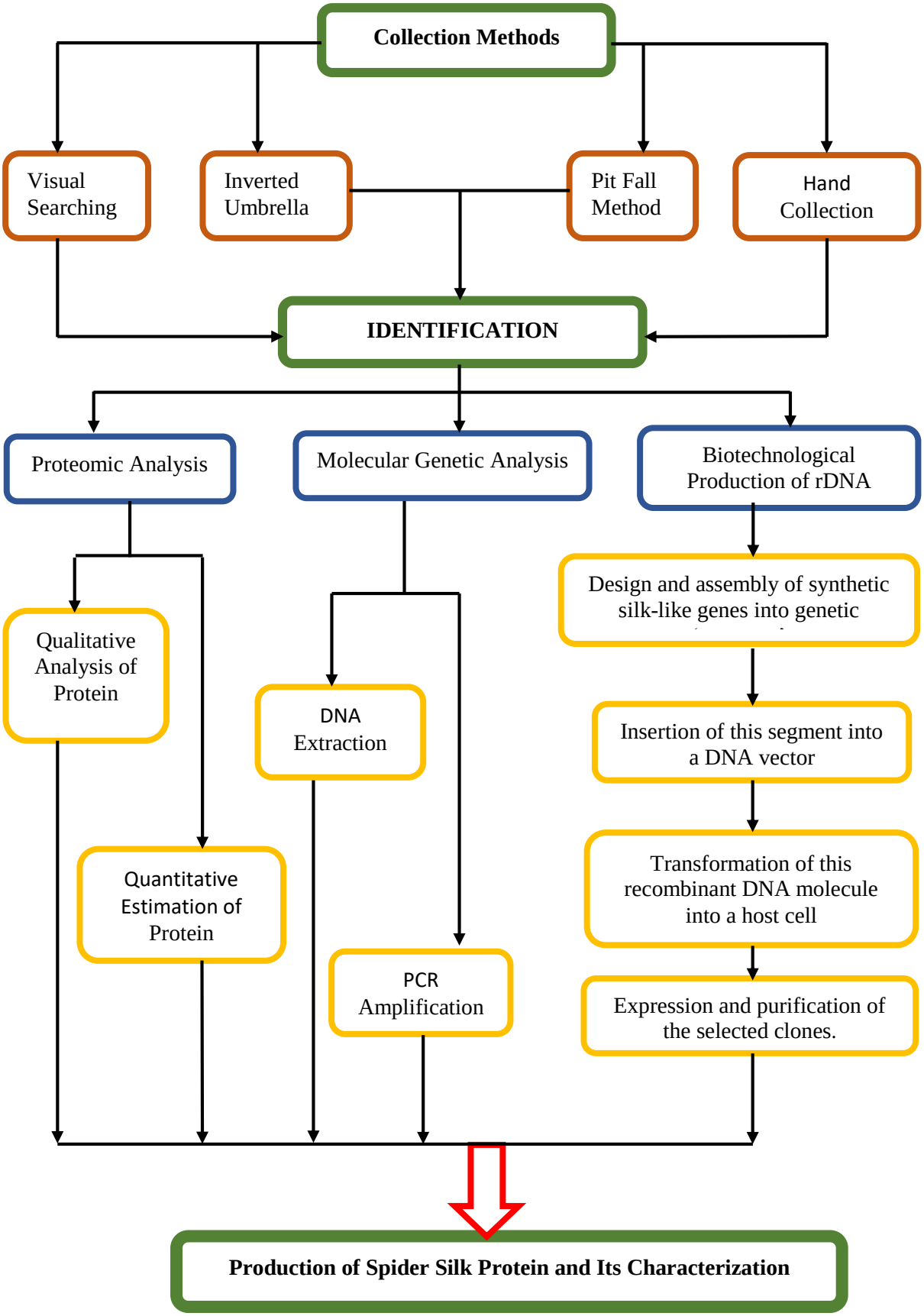
Collection Methods
Visual Searching
Inverted Umbrella
Pit Fall Method
Hand Collection
IDENTIFICATION
Proteomic Analysis
Molecular Genetic Analysis
Biotechnological Production of rDNA
Qualitative Analysis of Protein
Quantitative Estimation of Protein
DNA Extraction
PCR Amplification
Design and assembly of synthetic silk-like genes into genetic
Insertion of this segment into a DNA vector
Transformation of this recombinant DNA molecule into a host cell
Expression and purification of the selected clones.
Production of Spider Silk Protein and Its Characterization

Fig.1. Plan of Work

5.0. REFERENCES

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